

WHAT IS CLAIMED IS:

1 1. An isolated nucleic acid encoding an antifreeze protein, said protein
2 defined as follows:

- 3 (i) having a calculated molecular weight of between 7 and 13
4 kDa;
5 (ii) having a thermal hysteresis activity greater than about 1.5°C
6 at about 1 mg/mL; and
7 (iii) (a) specifically binding to an antibody raised against
8 antifreeze proteins selected from the group consisting of YL-1, YL-2, YL-3 or YL-4; or
9 (b) having at least 60% amino acid sequence identity to
10 antifreeze proteins selected from the group consisting of YL-1, YL-2 YL-3 or YL-4; or
11 (c) having an amino acid sequence comprising at least
12 one repeating unit of the consensus sequence of SEQ ID NO: 1.

1 2. The isolated nucleic acid of claim 1, wherein the calculated molecular
2 weight of the encoded protein is between about 8 and 12 kDa.

1 3. The isolated nucleic acid of claim 1, wherein the thermal hysteresis
2 activity is greater than 2°C at 1 mg/mL.

1 4. The isolated nucleic acid of claim 1, wherein the encoded protein has
2 at least 80% sequence identity to antifreeze proteins selected from the group consisting of
3 YL-1, YL-2, YL-3 and YL-4.

1 5. The isolated nucleic acid of claim 1, wherein the encoded protein is
2 selected from the group consisting of YL-1, YL-2, YL-3, and YL-4.

1 6. The isolated nucleic acid of claim 1, wherein the encoded protein
2 consists of about 5 to 12 repeating units of at least 5 out of 6 conserved amino acids of
3 SEQ ID NO: 1.

1 7. An isolated nucleic acid which specifically hybridizes to SEQ ID
2 NO:2 under stringent conditions.

1 8. An isolated nucleic acid encoding at least 1 repeat of a 12 contiguous
2 amino acid motif from a purified *Tenebrio molitor* antifreeze protein which specifically
3 binds to an antibody directed against antifreeze proteins selected from the group consisting
4 of YL-1, YL-2 YL-3 and YL-4

1 9. An isolated antifreeze protein, said protein:
2 (i) having at least 1 repeat of a 12 contiguous amino acid motif
3 comprising SEQ ID NO:1;
4 (ii) having a calculated molecular weight of about 7.0 to 13.0
kDa;
5 (iii) having a pI of about 8.0 to 10.0;
6 (iv) having a thermal hysteresis activity of greater than about
7 1.5°C at about 1 mg/mL; and
8 (v) (a) having the amino acid motif as shown in SEQ ID
9 NO:3 at the N-terminus; or
10 (b) specifically binding to antibodies raised to antifreeze
11 proteins selected from the group consisting of YL-1, YL-2, YL-3 and YL-4; or
12 (c) having 60% amino acid sequence identity to
13 antifreeze proteins selected from the group consisting of YL-1, YL-2, YL-3 and YL-4.
14

1 10. The isolated antifreeze protein of claim 9, wherein the number of
2 repeating motifs is from 5 to 12.

1 11. The isolated antifreeze protein of claim 9, wherein the thermal
2 hysteresis activity is greater than about 2.0°C at a concentration of about 1 mg/mL.

1 12. The isolated antifreeze protein of claim 9, wherein the protein is
2 selected from the group consisting of YL-1, YL-2, YL-3 and YL-4.

3 13. An antibody, specifically immunoreactive under immunologically
4 reactive conditions, to an antifreeze protein, said protein having at least 1 repeat of a
5 sequence comprising the motif of SEQ ID NO:1.

1 14. The antibody of claim 13, wherein the antifreeze protein further
2 comprises the amino acid sequence SEQ ID NO:4.

1 15. An organism into which, or into an ancestor of which, an exogenous
2 nucleic acid sequence which specifically hybridizes under stringent conditions to SEQ ID
3 NO:4 has been introduced and the organism translates the exogenous nucleic acid into an
4 antifreeze protein.

1 16. The organism of claim 15, wherein the exogenous nucleic acid
2 sequence is translated into an antifreeze protein which is expressed externally from the
3 organism.

17. The organism of claim 15, wherein said organism is a fish.

18. The organism of claim 18, wherein said fish is a member of the
family Salmonidae.

19. The organism of claim 15, wherein said organism is a crustacean.

1 20. The organism of claim 19, wherein said organism is a member of the
2 suborder Natantia.

1 21. The organism of claim 20, wherein said organism is selected from the
2 group consisting of the genera *Palaemonetes* and *Penaeus*.

1 22. The organism of claim 19, wherein said organism is a member of the
2 genus *Homarus*.

- 1 23. The organism of claim 15, wherein said organism is a plant.
- 1 24. The organism of claim 15, wherein said organism is a fungus.
- 1 25. The organism of claim 24, wherein the fungus is a yeast.
- 1 26. The organism of claim 25, wherein the yeast is selected from the
2 group consisting of *Torulopsis holmii*, *Saccharomyces fragilis*, *Saccharomyces cerevisiae*,
3 *Saccharomyces lactis*, and *Candida pseudotropicalis*.
27. The organism of claim 15, wherein the organism is a bacteria.
28. The organism of claim 27, wherein the bacteria is selected from the
2 group consisting of *Streptococcus cremoris*, *Streptococcus lactis*, *Streptococcus*
3 *thermophilus*, *Leuconostoc citrovorum*, *Leuconostoc mesenteroides*, *Lactobacillus*
4 *acidophilus*, *Lactobacillus lactis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, and
Bifidobacterium longum.
29. A method for decreasing the freezing point of an aqueous solution,
2 said method comprising the addition of an antifreeze protein having greater than 1 repeat of
3 the contiguous amino acid motif of SEQ ID NO:1, to said aqueous solution.
- 1 30. The method of claim 29, wherein the aqueous solution is applied to
2 an organism.
- 1 31. The method of claim 29, wherein the number of repeating motifs is
2 between about 5 to about 12.
- 1 32. The method of claim 29, wherein the antifreeze protein is produced
2 by recombinant means.

1 33. The method of claim 29, wherein the antifreeze protein further
2 specifically binds to an antibody directed against YL-1, YL-2, YL-3 or YL-4.

1 34. The method of claim 29, wherein the antifreeze protein is selected
2 from the group consisting of YL-1, YL-2, YL-3, and YL-4.

1 35. The method of claim 32, wherein the antifreeze protein is encoded by
2 a nucleic acid molecule which specifically hybridizes to SEQ ID NO:2.

add
a₁₁